Source specific cyto- and genotoxicity of atmospheric aerosol samples

Journal:	*Aerosol and Air Quality Research*
Manuscript ID:	AAQR-15-03-SIIAC-0131.R1
Manuscript Type:	special issue: 2014 International Aerosol Conference
Date Submitted by the Author:	07-Aug-2015
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Keywords:	PM2.5, Source Apportionment, Toxicology

We determined cyto- and genotoxicity of PM2.5 samples.
We performed on-line source apportionment based on Aethalometer measurement.
We measured OC/EC and heavy metal content of PM 2.5 samples.
We revealed connection between emission source and cyto- and genotoxicity.

Source specific cyto- and genotoxicity of atmospheric aerosol samples

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Abstract

Atmospheric aerosol samples were studied during wintry conditions at three Hungarian locations (rural background, urban background, traffic site). Ratio of biomass burning and fossil fuel related aerosol were highly different at the sampling points. Cyto- and genotoxicity of the samples were measured by using *Pseudomonas putida* growth inhibition test and Ames test, respectively. Dominant particle emission sources were apportioned through tracer heavy metal content measurement, optically and thermo-optically methods. According to the results, both ecotoxicity parameters are strongly emission source dependent; the higher the ratio of the biomass burning related carbonaceous aerosol the higher the cytotoxicity and the higher the ratio of the fossil fuel related carbonaceous aerosol the higher the genotoxicity. Cytotoxicity showed positive correlation with carbonaceous aerosol related to biomass burning (R^2=0.74) and negative with lead content of the samples (R^2=-0.56). Genotoxicity showed positive correlation with carbonaceous aerosol related to traffic (R^2=0.42) and cadmium content of the samples (R^2=0.74). At the same time, it showed negative correlation with organic/elemental carbon ratio of the samples (R^2=-0.43).

***Keywords*:** PM2.5, Source Apportionment, Toxicology

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INTRODUCTION

Identification of atmospheric aerosol emission sources is one of the most challenging topics in~~of~~ environmental science. The Clean Air for Europe (CAFE) Program, which exists within the 6th Environment Action Programme, claims that atmospheric aerosols are among the most dangerous air pollutants. Atmospheric particulate matter (PM) contains various carcinogenic and mutagenic compounds. It is generally accepted that these compounds can cause respiratory diseases such as lung cancer. Traffic-related sources such as vehicular exhaust systems, brake or tire wear and biomass burning are significant emitters of problematic aerosol substances. Daily average of the traffic related emission is much more constant ~~The traffic sources emit more or less constant amounts of PM~~ throughout the year ~~while~~ then the biomass burning source ~~that is strongly seasonal~~ (Wehner and Wiedensohler, 2003). Extensive public health studies have established the link between mass concentrations of PM2.5/PM10 and health problems within the population (Pope and Dockery, 2006 and references therein). However, there is a lack of direct measurements of the particle-based toxicological hazard of aerosols due to the low concentration and the chemical complexity of the PM2.5/PM10 (Steenhof et al., 2011; Soto et al., 2008). It is assumed that only a small fraction of combustion aerosol species is harmful. One of the most important pollutants is polycyclic aromatic hydrocarbons (PAHs). ~~,~~ Under specific traffic conditions,~~ial~~ pollutants like heavy metals can be occurred (de Kok *et al.*, 2005). Both of these processes are accompanied with black carbon (BC) emissions, for which it was shown that it is better correlated with public health effects compared to the concentration of sulphates, nitrates or PM10 (Atkinson *et al.*, 2014; Jansen *et al.*, 2012).

The most common source apportionment methods are the chemical mass balance (CMB) technique (Hedberg *et al.*, 2006; Schauer and Cass 2000, Schauer *et al.*, 2007, Watson,

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1984~~, Hedberg et al., 2006~~) and on-line Aerosol Mass Spectrometer (AMS) measurements combined with positive matrix factorization (PMF) (Lanz *et al.*, 2007 and 2008). Radiocarbon measurements (Currie *et al.*, 1994; Szidat *et al.*, 2006 and 2007) and the "Aethalometer model"~~, which is based on the measurement of aerosol light absorption at different wavelengths (F~~ (Favez *et al.*, 2010; Kirchstetter *et al.*, 2004; Sandradewi *et al.*, 2008~~, Favez et al., 2010~~), are also frequently used to distinguish between wood combustion and other sources. Although optical absorption-based methods (for example photoacoustic spectroscopy or Aethalometer) measure only the light absorbing fraction of the total PM, several studies demonstrated the connection of the apportioned sources with the results of other models. Favez and coworkers (2010) demonstrated a very good consistency between temporal variations obtained from CMB (performed with off-line filter measurements), PMF (applied to AMS measurements), as well as using the "Aethalometer model". Utry *et al.* (2014) established connection between optics-based source apportionment (from multi-wavelength photoacoustic measurement) and as well concentration of gaseous components (NO_x and CO), as un-carbonaceous constituents of the particles (K, Ca, Fe, Si). Source apportionment of BC used in this study does not provide total mass of aerosols produced by traffic and biomass burning but predict~~ions~~ the amount of soot produced by each of the two combustion sources.

Though *Pseudomonas putida* growth inhibition test is typically used for examination of toxicity in soil, sediment, surface water and groundwater samples, several studies demonstrated that it is also suitable to detect pollutants which are present in the air and is bounded to the surface of the PM fraction. This bacterium is aerob and unable to grow without the appropriate functioning of the dissimilatory system took place in the cytoplasmic membrane. Any type of pollutant disturbing the membrane integrity or inhibitory to the electron transport chain inhibit the metabolism, and as a consequence the growth of the bacterium will be retarded. Hence, this bacterial test system is an adequate method for air

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pollution testing as it sensitively detects heavy metals, phenol derivatives, nitroaromatics and PAH-s (Hahna *et al.*, 2007; Teodorovic *et al.*, 2009; van Beelen and Fleuren-Kemila, 1997; Vodovnik *et al.*, 2012).

For the fast genotoxicity investigations of aerosol samples, the SOS chromotest (Quillardet *et al.*, 1982) and distinct variants of Ames test (Gatehouse, 2012) or their combinations (Škarek *et al.*, 2007) are the most frequently used methods. Shortly after the development of a sensitive microbiological assay for genotoxicity by Ames (1975), Pitts *et al.* (1977) used the Ames assay system for investigating mutagenic activity in the organic fraction of ambient airborne particulates. Škarek *et al.* (2007) investigated the genotoxicity of organic extracts of total suspended particles (TSP) and PM2.5 with SOS chromotest. The results of the bioassays indicated potential health risks for the population exposed to the organic air pollutants, especially at the urban localities. The relationship between the genotoxicity of atmospheric samples and particle size were studied by Kawanaka *et al.* (2004) and ~~,~~ by Boschini *et al.* (2001) with Ames plate test (TA98 and TA100 strains, with or without S9 fraction treatments), gene conversion and reversion ~~–~~in the *Saccharomyces cerevisiae* D7 strain~~,~~ and comet assay on human leukocytes. The PM2.5 fraction of airborne particulate generally showed the highest DNA-damaging activity. Nordina *et al.* (2015) investigated the influence of ozone initiated atmospheric processing on the physicochemical and toxicological properties of particulate emissions from wood combustion. The collected PM was investigated toxicologically *in vitro* with a mouse macrophage model. DNA damage was assessed by the alkaline single cell gel electrophoresis (comet assay). The ecotoxicity differences of artificial emission samples and ambient aerosol samples were shown using a method based on the *Vibrio fischeri* bioluminescence inhibition bioassay (Turoczi *et al.*, 2012). However, the genotoxicity of aerosols from different sources has not been studied.

The aim of this paper is the investigation of the potential connection between toxicity

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and different source specific parameters (i.e. organic carbon/elemental carbon, fossil fuel and biomass burning related components of BC and heavy metals) of atmospheric samples. Beside genotoxicity tests based on Ames method, *Pseudomonas putida* growth inhibition test was applied for cytotoxicity determination of aerosol filter extracts. A pre-processing method was also developed that allows toxicological testing of standard PM2.5/PM10 samples for both Ames test and *P. putida* growth inhibition test. This study presents the application of this method on PM2.5 samples collected from different sampling points.

METHODS

Measurement sites

PM2.5 samples were collected on a 24 h basis on pre-baked Whatman quartz filters at three different measurement sites (rural background, urban background, roadside) during wintry conditions. Average PM10 mass concentration during the sampling periods was 20.9, 30.5 and 38.15 μg/m^3, respectively. In total, 52 samples were collected.

Site 1 is the rural background station K-puszta, which is located in a clearing in a mixed forest on the Hungarian Great Plain in the middle of the Carpathian Basin. The nearest large city is Kecskemét (population 110,000), located 15 km southeast from the station. The nearest major pollution source in the prevailing wind direction (northwest) is Budapest (population 1.9 million), approximately 70 km from the station. PM2.5 samples were taken between 11/01/2013 and 08/02/2013 using a high volume sampler in the framework of an intensive EMEP campaign.

Site 2 is an urban background site located in a schoolyard in a residential area of Kecskemét, Hungary. PM 2.5 samples were collected between 14/11/2013 and 27/11/2013 using a Digitel high volume sampler.

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Site 3 is a traffic site located 300 m from the highway 5 (Tóth László walkway, Kecskemét) linking the city centre of Kecskemét to motorway 5 (distance of 5 km). The annual average of the total motorized traffic at this junction is about 1500 vehicles/hour. PM2.5 samples were collected between 08/03/2014 and 19/03/2014 using a Digitel high volume sampler.

Optics-based source apportionment

Source apportionment of BC emissions using Aethalometer measurements is based on the model of Sandradewi *et al.* (2008), with optical absorption coefficient (b_{abs}) being a sum of biomass burning (bb) and fossil fuel (ff) burning fractions:

$$b_{abs}(470\ nm) = b_{abs}(470\ nm)_{ff} + b_{abs}(470\ nm)_{bb} \quad (1)$$

$$b_{abs}(950\ nm) = b_{abs}(950\ nm)_{ff} + b_{abs}(950\ nm)_{bb} \quad (2),$$

where $b_{abs}(\lambda)$ is the absorption coefficient at wavelength λ. The model is based on the difference in the wavelength dependence of the absorption coefficients ~~of~~for aerosols from ~~both~~ the two sources; it is assumed that the absorption coefficients of aerosols from fossil fuel and biomass ~~combustion~~ burning described with Ångström's law ~~with Ångström exponents α_{ff} and α_{bb}~~ are:~~:~~

$$\frac{b_{abs}(470\ nm)_{ff}}{b_{abs}(950\ nm)_{ff}} = \left(\frac{470}{950}\right)^{-\alpha_{ff}} \quad (3)$$

$$\frac{b_{abs}(470\ nm)_{bb}}{b_{abs}(950\ nm)_{bb}} = \left(\frac{470}{950}\right)^{-\alpha_{bb}} \quad (4),$$

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where α_{ff} and α_{bb} are the Ångström exponents related to fossil fuel and biomass burning, respectively. Solving ~~equation~~Eqs. (1-4) enables the calculation of the biomass burning and fossil fuel related BC fractions:

$$\frac{BC_{bb}}{BC} = \frac{b_{abs}(950\ nm)_{bb}}{b_{abs}(950\ nm)} \quad (5)$$

$$\frac{BC_{ff}}{BC} = \frac{b_{abs}(950\ nm)_{ff}}{b_{abs}(950\ nm)} \quad (6).$$

BC measurements were performed using a seven-wavelength Aethalometer model AE33 (Drinovec *et al.*, 2014). Ångström exponent values of α_{ff}=1 for fossil fuel and α_{bb}=2 for biomass have been used for source apportionment.

Toxicity testings

The filter extracts were made from 1 cm^2 filter pieces with sterile distilled water in Eppendorf-tubes agitated with sterile glass beads in a high frequency Eppendorf-tube shaker. After centrifugation the supernatants were used for further processing. These extracts were centrifuged through a cellulose acetate membrane (pore size: 0.22 μm) containing spin column (Corning® Costar® Spin-X® centrifuge tube filters, Sigma).

Cytotoxicity determination

For the cytotoxicity investigation the *Pseudomonas putida* growth inhibition test (ISO 10712:1995) was used, adapted to 0.2 ml end volume in microtiter plate wells. The optical density of mini-cultures was followed with a microtiter plate photometer.

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Genotoxicity investigations

A new microtiter plate version of the Ames test (Ames *et al.*, 1975) was developed and used in this work. *Salmonella typhimurium* histidine auxotrophic mutant strains (TA98 and TA1535) were used in this test. The *Salmonella* strains were grown in LB (Luria-Bertani) medium for 1 day at 37 °C. LB bacterial culture medium (Bertani, 1952) contains 10 g/l bacto trypton, 5 g/l yeast extract and 10 g/l NaCl. The *Salmonella* cells were pelleted from the cultures by centrifugation and resuspended in minimal liquid medium (Mortelmans and Zeiger, 2000). The optical density of the suspensions was set to 0.5 at 620 nm by dilution with minimal medium. A mixture of 0.15 ml of bacterium suspension and 0.05 ml filtered aerosol extract was applied to each well of the microtiter plate. The optical density of microcultures was measured at 620 nm using a microtiter plate photometer before and after 48 hour of incubation. The measured optical density increase was in strong positive correlation with the number of the revertants and so with the genotoxicity of the samples.

Determination of chemical composition

The organic and elemental carbon content (OC and EC, respectively) of the PM2.5 samples was measured using a thermo-optical method with a Sunset Lab OCEC Aerosol Analyser with EUSAAR 2 protocol (Cavalli *et al.*, 2010). Heavy metal content of the samples was measured by atomic absorption spectroscopy according to MSZ21454/6-86 Hungarian standard.

RESULTS AND DISCUSSION

Our novel sample pre-processing method ensures an efficient sterile extraction of particulate matter from filters into the solution. An important task was the removal of the heat and radiation resistant *Bacillus* spores which are present in substantial amounts on the filters.

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Instead of heat or radiation treatments – which could cause undesired chemical reactions in the samples – the extracts were centrifuged through a cellulose acetate membrane filter with 0.22 µm pore size (Corning® Costar®Spin-X®centrifuge tube filters, Sigma).

All measured raw data are collected in Table 1, averaged pertaining to the three sampling points. Mass concentration of PM10 was increasing properly as expected (lowest at the background station – Site 1 and doubled at the traffic site – Site 3). While the maximum of the mass concentration was the lowest at Site 1, the maximum of the BC concentration and cytotoxicity (*Pseudomonas* growth inhibition – PS) were the highest. The extremely high, - even exceeding the air quality limit value -, PM10 maximums at Site 2 and 3 did not show any connection with the toxicity values. The mass concentration of cadmium (Cd), originating from traffic emission (Terzi *et al.*, 2010), was almost three times higher at Site 3 than at Site 2 (rural background). In case of lead, originating mostly from wheel weights (Salma & Maenhaut, 2006), the increase at Site 3 can be noticed only if mass of the total sample is taken into consideration.

In order to eliminate the disturbance of the different mass of the single particle samples (or the mass concentration in case of in-situ measurement) we calculated mass normalized ratios from the determined source related quantities such as OC/EC, BC_{ff}/BC and BC_{bb}/BC. These values are already independent of the amount of the sample and are connected to the type of the pollution. Correlation coefficients between the measured toxicological and source specific parameters (determined by the least squares method) are summarized in Table 2~~1~~. Connections having p-values lower than 10^{-3} (labelled with asterisk in Table 2~~1~~) were studied.

In case of optics-based source apportionment, we found a very high biomass burning contribution at Site 1 (BC_{bb}/BC as high as 60 %) and a strong connection between the biomass burning related fraction of BC and cytotoxicity (PS) (Fig. 1(~~(~~a)~~)~~). PS did not show

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any correlation with fossil fuel related BC fraction. On the other hand, traffic was usually quite high at Site 3 and always low at Site 2. The fossil fuel fraction of BC showed a reliable correlation with genotoxicity measured with the TA98 strain (Fig. 1(b)), but no significant connection with genotoxicity determined with the TA1535 strain. The source apportion method based on optical measurements depends on the increased organic aerosol content produced by incomplete biomass combustion. The correlation of cytotoxicity with the biomass burning related fraction of BC is supported by the higher toxicity of incomplete combustion aerosols (Bolling *et al.*, 2009).

Results of heavy metal content analysis confirmed our previous findings. PS showed negative correlation with lead concentration (~~originating mostly from wheel weights (Salma & Maenhaut, 2006);~~ Fig. 2(a)). Genotoxicity determined with the TA1535 strain correlated positively and strongly with concentration of cadmium ~~originating from traffic emission (Terzi et al., 2010;~~ (Fig. 2(b)). There was no correlation between genotoxicity measured with the TA98 strain and any measured heavy metal component. De Kok *et al.* (2005) showed that traffic emission genotoxicity is most closely correlated with both PAH and metal content of the particles.

High OC/EC ratios can be indicative for the high contribution of biomass burning emissions (Soto-García *et al.*, 2011). OC/EC shows non-significant positive correlation with cytotoxicity and negative correlation with genotoxicity using TA98 strain (Fig. 3). This is in agreement with the results of the optics-based source apportionment results where high fossil fuel related BC content correlates with genotoxicity and biomass burning related BC correlates with the cytotoxicity. This can be understood by toxic effect of wood smoke being ascribed to the organics fraction of aerosols (Kocbach *et al.*, 2008).

CONCLUSIONS

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The ecotoxicity of aerosol samples collected during three winter time field campaigns on quartz fibre filters ~~were~~ was measured using a novel sample pre-processing method. Optical, thermo-optical and heavy metal analyses were used to indicate ~~major sources of these~~the ratio of traffic and biomass burning related fraction of winter time aerosol samples. The results ~~showed~~ indicate that genotoxicity of atmospheric aerosol samples is more closely related to traffic sources whereas~~and~~ cytotoxicity of the same PM2.5 samples is ~~related to~~better correlated with the biomass burning sources as determined by~~using~~ optically based source apportionment method.

ACKNOWLEDGEMENTS

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 “National Excellence Program”.

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407 ## List of Table captions

408

409 **Table 1:** Measured raw data pertaining to the three sampling locations

410 **Table 2:** Correlation coefficients between cytotoxicity (PS) and genotoxicity (TA98 and

411 TA1535) test results and selected aerosol parameters.

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412 # List of Figure Captions

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414 **Figure 1(a-b):** Correlation between optics-based source apportionment and toxicity of PM2.5
415 samples

416 **Figure 2(a-b):** Correlation between heavy metal compounds and cytotoxicity of PM2.5
417 samples

418 **Figure 3:** Correlation between Organic/Elemental carbon ratio and relative genotoxicity of
419 PM2.5 samples

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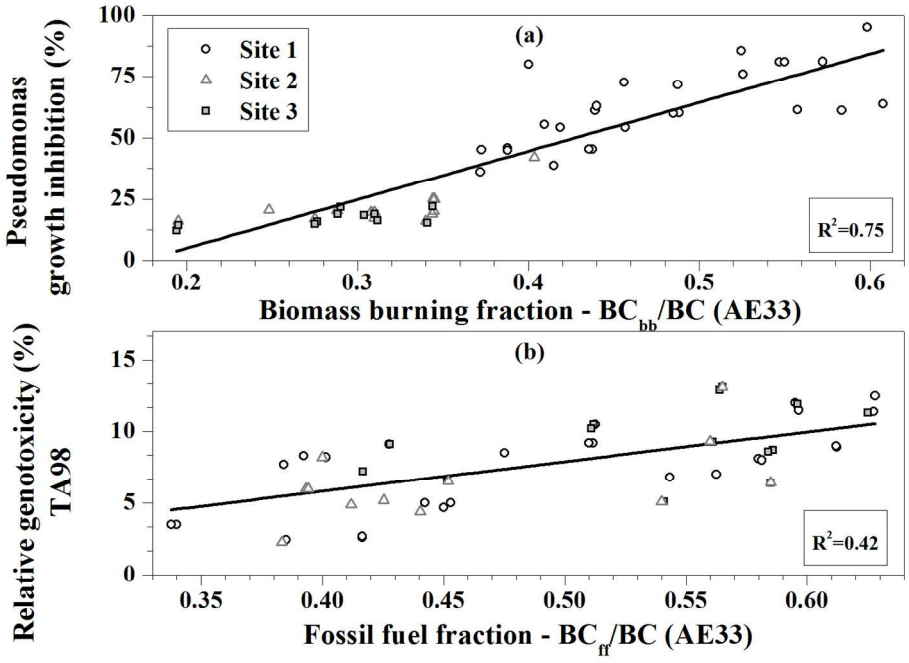
Table 1: Measured raw data pertaining to the three sampling locations

	Site 1 (N=26)			Site 2 (N=14)			Site 3 (N=12)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max
PM10 ($\mu g/m^3$)	20.9±10.25	8.44	39.96	30.54±14.26	11	62.7	38.15±15.14	16.58	64.25
OC ($\mu g/m^3$)	8.47±2.99	3.57	13.49						
EC ($\mu g/m^3$)	0.63±0.28	0.17	1.24						
BC ($\mu g/m^3$)	2.07±1.01	0.63	3.91	1.47±1.08	0.32	3.45	2.4±1.57	0.58	5.08
Pb (ng/m^3)				19.21±2.39	16.15	25.14	17.18±3.12	12.25	22
Cd (ng/m^3)				5.16±2.58	1	9.2	16.57±6.48	3.5	24.7
PS (%)	63.65±16.37	36.62	95.1	21.06±6.72	16.08	42.2	17.18±3.12	12.15	22
TA 98 (%)	7.51±3.00	2.47	12.5	9.34±2.47	5.1	13.1	6.45±2.75	2.3	13.1

Table 2: Correlation coefficients between cytotoxicity (PS) and genotoxicity (TA98 and TA1535) test results and selected aerosol parameters.

	BC_{ff}/BC	BC_{bb}/BC	OC/EC	Pb	Cd
PS	0.03	**0.74***	0.10	**-0.56***	0.27
TA98	**0.42***	-0.03	**-0.43***	0.08	-0.04
TA1535	0.32	-0.07	**--**	0.24	**0.74***

* $p<0.001$



Correlation between optics-based source apportionment and toxicity of PM2.5 samples
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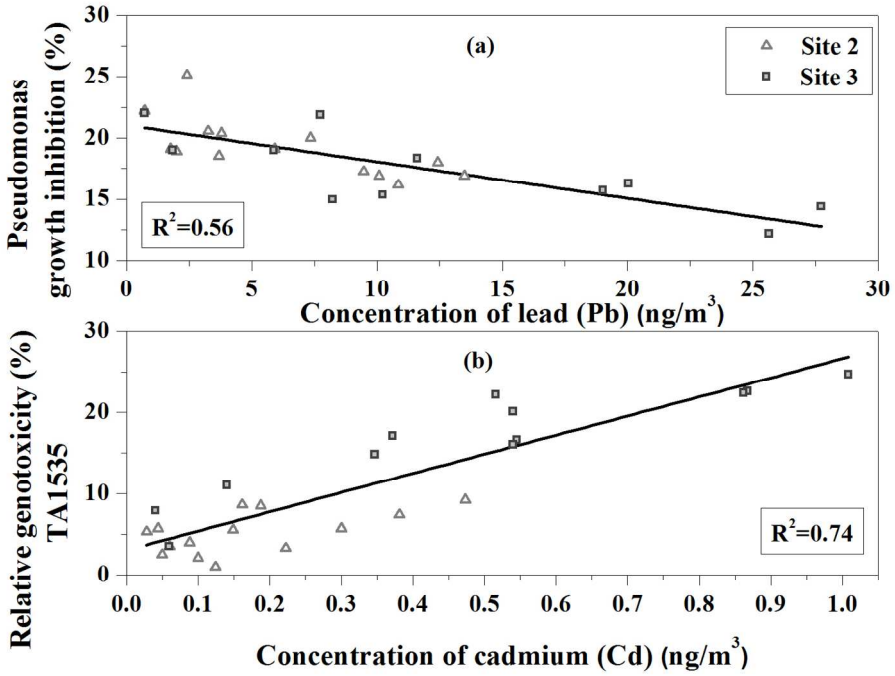

Correlation between heavy metal compounds and cytotoxicity of PM2.5 samples
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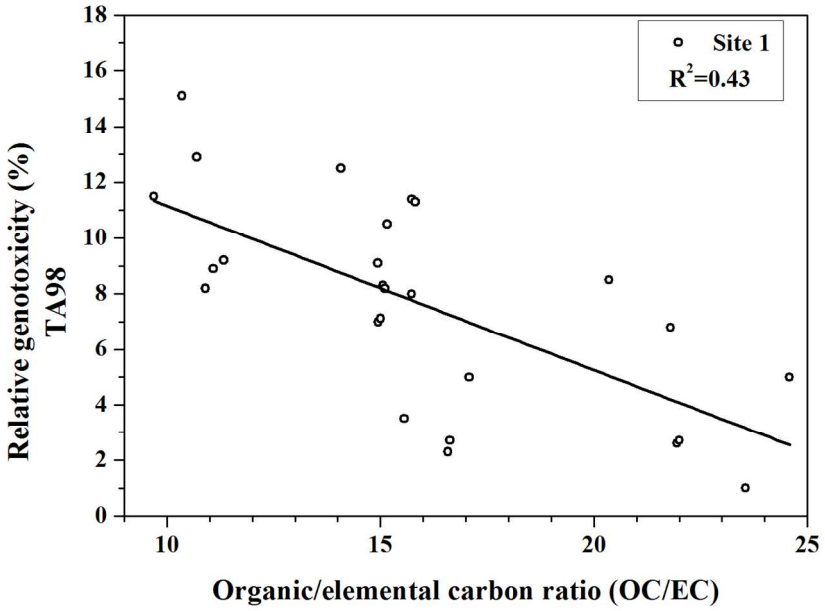

Correlation between Organic/Elemental carbon ratio and relative genotoxicity of PM2.5 samples
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